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DOCUMENT-IDENTIFIER: US 6475723 B2
TITLE: Pathogenic tau mutations

Abstract Text (1):
Isolated nucleic acid molecules that include a tau gene sequence are described. The tau gene sequences have a mutation linked to a Tau pathology. Transgenic non-human mammals containing a Tau pathology also are also described.

Brief Summary Text (2):
The invention relates to an isolated nucleic acid molecule that includes a tau gene sequence, wherein the tau gene sequence contains a mutation linked to a Tau pathology.

Brief Summary Text (6):
The invention is based on the discovery of mutations in the tau gene that are linked to Tau pathologies. Thus, the invention provides nucleic acid molecules that include such mutations, allowing animal models of neurodegenerative diseases to be developed. Identification of the mutations also provides methods for determining a diagnosis of neurodegenerative disease in a patient.

Brief Summary Text (7):
The invention features an isolated nucleic acid molecule including a tau gene sequence, wherein the molecule has a mutation linked to a Tau pathology. The nucleic acid molecule can be from about 15 nucleotides in length to full-length. The mutation can be located in an exon or in an intron. A mutation can be in exon 7, exon 9, exon 10, or in exon 13 and in particular embodiments, at a region encoding amino acids 152, 257, 272, 301, 389, or 406. In one embodiment, the mutation at amino acid 152 is a change from an alanine to a threonine residue, the mutation at amino acid 257 is a change from a lysine to a threonine residue, and the mutation at amino acid 272 is a change from a glycine residue to a valine residue. The mutation at amino acid 301 can be a change from a proline residue to a leucine residue. The mutation at amino acid 389 can be a change from a glycine to an arginine residue. The mutation of amino acid 406 can be a change from an arginine to a tryptophan residue. An additional mutation can include deletion of amino acid 280. The mutation also can be in a splice donor site region and, in a particular embodiment, can destabilize a stem-loop structure of the splice donor site region and can be in a region 13-16 nucleotides 3′ of the exon 10 splice donor site.

Brief Summary Text (9):
The invention also features a transgenic non-human mammal including a nucleic acid construct. The nucleic acid construct includes a regulatory nucleic acid sequence operably linked to a nucleic acid sequence encoding a Tau polypeptide. Expression of the Tau polypeptide is linked to a Tau pathology in the transgenic non-human mammal. The transgenic non-human mammal can be a rodent, and in particular, a mouse. The regulatory nucleic acid sequence can be a brain-specific promoter. In one embodiment, the Tau polypeptide is human Tau polypeptide, and can be wild-type or can contain a mutation linked to a Tau pathology. The mutation can be, for example, at amino acid 152, 257, 272, 280, 301, 389, or 406. In other embodiments, the transgenic non-human mammal also includes a nucleic acid construct that includes a regulatory sequence operably linked to a nucleic acid sequence encoding a human amyloid precursor protein or a human presenilin-1 protein.

Brief Summary Text (10):
The invention also relates to a method for determining a diagnosis, prognosis, or risk of neurodegenerative disease in a patient. The method includes detecting a tau gene mutation in genomic DNA of the patient, wherein the mutation is linked to a Tau pathology. Mutations that are linked to Tau pathologies are described above.

Drawing Description Text (4):
FIG. 1B is a schematic representation of the tau gene (exons 9-13) displaying the relative locations of missense mutations in the coding region and in the splice donor site. The effect of two missense mutations, G272V and P301L, on the PGGG motif in the microtubule binding domains encoded by exon 9 and 10, respectively, is shown.

Drawing Description Text (5):
FIG. 1C is a sequence alignment of the microtubule binding domain encoded by exon 10 with the equivalent regions from mouse and cow and from the human and rat microtubule associated protein 4 (MAP4). The location of the P301L missense mutation is indicated above the alignment.

Detailed Description Text (3):
In one aspect, the invention features an isolated nucleic acid molecule including a tau gene sequence, wherein the nucleic acid molecule has at least one mutation linked to a Tau pathology. As used herein, "isolated" refers to a sequence corresponding to part or all of the tau gene, but free of sequences that normally flank one or both sides of the tau gene in a mammalian genome. An isolated nucleic acid molecule can be, for example, a recombinant DNA molecule, provided one of the nucleic acid sequences normally found flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, isolated nucleic acid molecules include, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an

isolated nucleic acid molecule can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid molecule.

Detailed Description Text (6):
As used herein, "Tau pathology" refers to neurofibrillary tangles observed in brain, including one or more of the following: paired helical filaments (PHFs), straight Tau filaments, and any other type of Tau filament. Tau pathology also includes neuronal and/or glial inclusions or insoluble deposits that stain positively with anti-Tau antibodies. The tau gene encodes the microtubule associated protein Tau that is present in abnormal inclusions seen in FTDP-17 and is also the major component of the PHFs that make up the characteristic tangles seen in Alzheimer's disease (AD) and other neurodegenerative disorders. The human Tau protein found in brain is encoded by eleven exons. The sequence of the wild-type human tau gene is described by Andreadis, A. et al., Biochemistry, 31(43):10626-10633 (1992). The gene undergoes alternative splicing with exons 2, 3, and 10 absent from some forms of the mature brain tau mRNA. A total of six different tau mRNAs is generated as a result of this alternative splicing, with the mRNAs encoding proteins of 352-441 amino acids. The alternative splicing of exon 10 generates Tau protein with either three or four microtubule binding motifs that each are imperfect repeats of 31 or 32 residues. Tau protein containing three microtubule binding motifs is referred to herein as the three-repeat isoform, whereas Tau protein containing four microtubule binding motifs is referred to herein as the four-repeat isoform. Tau proteins containing three microtubule binding motifs (exon 10 -) form paired helical filaments, similar to those seen in the brains of AD patients, during in vitro aggregation experiments, while Tau proteins containing four microtubule binding motifs (exon 10+) form straight filaments.

Detailed Description Text (7):
The FTDP-17 locus maps to a 2 cM region on chromosome 17q21.1. As described herein, analysis of a large number of families with FTDP-17 and analysis of coding and non-coding regions of the tau gene revealed mutations linked to FTDP-17. The 11 tau coding exons and flanking intronic regions were initially sequenced in 38 individuals from families with fronto-temporal dementia from Scandinavia (9 families), the Netherlands (2 families), the USA (4 families), Australia (1 family), and from the Greater Manchester area of the UK (22 families). In this group, 7 families had previously displayed evidence for linkage to chromosome 17. The sequence analysis of the tau gene identified mutations in 5 of 7 families in which linkage to chromosome 17 had previously been indicated.

Detailed Description Text (9):
In exon 7, the mutation can include a change from an alanine to a threonine at residue 152 (i.e., A152T), which can result from a change at nucleotide 454 of a guanine to an adenine (i.e., G454A) in the tau gene sequence. A mutation in exon 9 from a lysine to a threonine at amino acid 257 (K257T) can result from a change of an adenine to a cytosine at nucleotide 770 of the tau gene sequence. Amino acid 272 can be changed from a glycine residue to a valine residue (G272V), a highly conserved residue within the microtubule

binding domain encoded by exon 9, and is found in all Tau isoforms. The G272V mutation can result from a change of a thymine to a guanine at nucleotide 815 of the tau gene sequence. Amino acid 389 can be changed from a glycine to an arginine residue (G389R) in exon 13, with a corresponding change from a guanine to an adenine at nucleotide 1166. Amino acid 406, a highly conserved residue near the carboxy terminus of Tau, can be changed from an arginine to a tryptophan residue (R406W), with a corresponding change from a cytosine to a thymine at nucleotide 1216 of the tau gene.

Detailed Description Text (11):
A deletion of amino acid 280 was detected in a single Dutch family, with corresponding nucleotides 838-840 deleted from the tau gene sequence. In addition, mutations were detected in the exon 10 splice donor site region. For example, heterozygous mutations were identified in a cluster of four nucleotides 13-16 bp 3' of the exon 10 donor splice site (FIG. 2). No mutations were found in the tau gene in two families (HDDD2 and HFTD3).

Detailed Description Text (12):
The mutations described herein appear to cause FTDP-17, one of the major autosomal dominant loci associated with neurodegeneration. These data also indicate that a tau gene variant (V337M) in a family with FTDP-17 (Seattle A), Spillantini, M. G. et al., Brain Path., 8:387-402 (1998), is likely pathogenic. The previous absence of mutations in other families had led to the suggestion that this might be a benign polymorphism. Most importantly, the identification of pathogenic missense and splice donor site mutations associated with FTDP-17 demonstrates for the first time that Tau dysfunction can lead to neurodegeneration. In addition, the nature of the splice-donor site mutations indicates that the relative levels of four-repeat and three-repeat Tau proteins are crucial to the correct functioning of Tau, at least in the brain. This is consistent with the observation that alternative splicing of exon 10 is known to be developmentally regulated. These mutations, by affecting the potential stem-loop structure in the exon 10 donor site, also reveal at least part of the mechanism by which alternative splicing of this exon is regulated. The existence of splice donor site, deletion, and missense mutations also may partially explain the variability observed in soluble Tau protein in FTDP-17.

Detailed Description Text (17):
Transgenic non-human mammals can be farm animals such as pigs, goats, sheep, cows, horses, and rabbits, rodents such as rats, guinea pigs, and mice, and non-human primates such as baboons, monkeys, and chimpanzees. Transgenic mice are particularly useful.

Detailed Description Text (21):
Expression of the nucleic acid sequence encoding a Tau polypeptide in the tissues of the transgenic non-human mammals can be assessed using techniques that include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse-transcriptase PCR (RT-PCR). A Tau pathology can be manifested as, for example, neurofibrillary tangles or neuronal and/or glial inclusions or insoluble deposits that stain positively with anti-Tau antibodies, as described above.

Anti-Tau antibodies are available commercially from, for example, Sigma Chemical Company (St. Louis, Mo.). Anti-Tau antibodies that are sensitive to phosphorylated epitopes of Tau are available from Innogenetics (Belgium).

Detailed Description Text (22):
In one embodiment, transgenic mice further include a nucleic acid construct encoding a human amyloid precursor protein (APP) or human presenilin-1 (PS-1) protein. The nucleic acid sequence of human APP has GenBank Accession No. D87675 g2429080. The nucleic acid sequence encoding human PS-1 protein has GenBank Accession Nos. L76518-L76528. Expression of APP or PS-1 can enhance the Tau pathology observed with expression of Tau polypeptides. The ratio of Tau polypeptide containing four microtubule binding motifs to Tau polypeptide containing three microtubule binding motifs (i.e, ratio of four-repeat to three-repeat isoform) plays a role in the correct functioning of the Tau protein. As described herein, the splicing mutations affect the relative levels of four-repeat Tau and three-repeat Tau proteins, which may have important implications for the modeling of Alzheimer's disease since mouse brains contain nearly all four-repeat Tau (almost the direct opposite of the human brain). The mouse tau gene lacks a "stable" stem-loop structure to regulate alternate splicing of exon 10 leading to the presence of a single isoform. Current transgenic mouse models of Alzheimer's disease expressing APP at high levels develop amyloid plaques (A.beta. plaques) and contain hyper-phosphorylated Tau polypeptide, but do not contain Tau positive tangles or exhibit significant cell loss.

Detailed Description Text (23):
Therefore, it appears that there is a difference in the response of mouse and human neurons to an amyloid insult. Given the evidence of Tau involvement in neurodegeneration, provided by the mutations described herein, and the dramatic difference in the ratio of four-repeat and three-repeat Tau isoforms in the mouse and human brains, Tau polypeptide seems a likely candidate to explain the difference in the response of mouse and human neurons to amyloid. This hypothesis is further strengthened by the observation, again from the splice site mutations described herein, that the ratio of four-repeat to three-repeat Tau is indeed important to Tau function. Disturbances in this ratio can lead to neurodegeneration. The fact that mice have a preponderance of four-repeat Tau isoform also may be significant since four-repeat Tau protein binds more tightly to microtubules (compared to three-repeat Tau) and produces more stable microtubules. It is possible therefore that the mouse is better able to withstand the process induced by amyloid (A.beta.) that leads to neurodegeneration and Alzheimer's disease in humans.

Detailed Description Text (24):
Transgenic mice expressing a Tau polypeptide and APP or PS-1 can be produced by crossing transgenic mice overexpressing human three-repeat tau cDNAs (wild type cDNAs or cDNAs containing pathogenic mutations) with mice overexpressing human APP and/or human PS-1 cDNAs. Transgenic mice overexpressing human APP or human PS-1 are described, for example, in WO 97/48792 and WO 97/27296, respectively. See, WO 98/17782 for a description of transgenic mice

containing mutant APP and mutant PS-1 transgenes. Alternatively, a single line of transgenic mice can be produced by initially preparing the mice using the appropriate constructs. Increasing the proportion of three-repeat Tau in the mouse brain may lead to greater susceptibility to amyloid insults, tangle formation, and cell death, generating an improved model of Alzheimer's disease and providing insight into the disease process.

Detailed Description Text (26):
In another aspect, the invention features a method for determining a diagnosis, prognosis, or risk of neurodegenerative disease in a patient. The method includes detecting a tau gene mutation in genomic DNA of the patient, wherein the mutation is linked to a Tau pathology. Neurodegenerative diseases, include, for example, FTDP-17, Pick's disease, Progressive Supranuclear Palsy (PSP), Cortico-basal degeneration (CBD), lytico and bodig disease of Guam, variants of AD with straight tau filaments, and any other neurodegenerative diseases in which Tau pathology is a major feature. Tau pathology includes neurofibrillary tangles observed in brain, including one or more of the following: paired helical filaments (PHFs), straight Tau filaments and any other type of Tau filament. Tau pathology also includes neuronal and/or glial inclusions or insoluble deposits that stain positively with anti-Tau antibodies.

Detailed Description Text (27):
Tau gene mutations can be detected by various methods. Mutations can be detected, for example, by sequencing exons and introns of the tau gene, restriction fragment length polymorphisms (RFLP) analysis, PCR-RFLP analysis, allele-specific hybridizations, mutation specific polymerase chain reactions (MSPCR), or by single stranded conformational polymorphism (SSCP) detection.

Detailed Description Text (28):
Genomic DNA is generally used in the detection of tau gene mutations. Genomic DNA typically is extracted from peripheral blood samples, but also can be extracted from, for example, mucosal scrapings of the lining of the mouth. Brain tissue obtained from an autopsy also can be used for post-mortem diagnosis. Routine methods can be used to extract genomic DNA from a blood or tissue sample, including, for example, phenol extraction. Alternatively, genomic DNA can be extracted with kits such as the QIAamp.RTM. Tissue Kit (Qiagen, Chatsworth, Calif.), Wizard.RTM. Genomic DNA purification kit (Promega, Madison, Wis.), and the A.S.A.P..TM. Genomic DNA isolation kit (Boehringer Mannheim, Indianapolis, Ind.).

Detailed Description Text (29):
For example, exons and introns of the tau gene can be amplified through PCR and then directly sequenced. This method can be varied, including using dye primer sequencing to increase the accuracy of detecting heterozygous samples. Alternatively, a nucleic acid molecule can be selectively hybridized to the PCR product to detect a gene variant. Hybridization conditions are selected such that the nucleic acid molecule can specifically bind the sequence of interest, e.g., the mutant nucleic acid sequence. Such hybridizations typically are performed under high stringency as many mutations include only a single nucleotide difference. High

stringency conditions can include the use of low ionic strength solutions and high temperatures for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1.times. SSC), 0.1% sodium dodecyl sulfate (SDS) at 60.degree. C. Alternatively, denaturing agents such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42.degree. C. Another example is the use of 50% formamide, 5.times. SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5.times. Denhardt's solution, sonicated salmon sperm DNA (50 .mu.g/ml), 0.1% SDS, and 10% dextran sulfate at 42.degree. C., with washes at 42.degree. C. in 0.2.times. SSC and 0.1% SDS. Hybridization conditions can be adjusted to account for unique features of the nucleic acid molecule, including length and sequence composition.

Detailed Description Text (33):
Alternatively, the ratio of exon 10+ and exon 10- RNA can be measured and used for determining a diagnosis, prognosis or risk of neurodegenerative disease in a patient. For example, reverse transcriptase PCR (RT-PCR) can be performed on total RNA isolated from a patient. After amplification, PCR products can be resolved into fragments with and without exon 10, and the molar ratio of exon 10+ to exon 10- RNA can be determined using densitometry. Patients with an increase in the proportion of exon 10+ RNA can have a mutation in the splice donor sites of the tau gene.

Detailed Description Text (38):
Tau Gene Sequencing

Detailed Description Paragraph Table (2):
TABLE 2 Families with segregating mutations within the tau gene Family Affected (PM Mean onset age ID Origin (founder) confirmed) Generations (years) Mutation HFTD2* Netherlands 34(15) 7 47 G272V HFTD1* Netherlands 49(14) 5 50 P301L FTD003 USA 3(2) 2 45-50 P301L Man19 UK 3(1) 2 65 Ex 10 splice + 13 DDPAC* Ireland 13(6) 3 44 Ex 10 splice + 14 AusI* Australia(UK) 28(5) 5 53 Ex 10 splice + 16 FTD002* USA 3(1) 2 40 Ex 10 splice + 16 Man6 UK 2(1) 1 48 Ex 10 splice + 16 Man23* UK 10(2) 3 51 Ex 10 splice + 16 FTD004 USA 10(2) 4 55 R406W HFTD4 Netherlands 2 2 53 (one patient) Deletion of K280 *Families that displayed prior evidence of genetic linkage to chromosome 17.

Other Reference Publication (3):
Hammer et al. Journal of Animal Science 63(1):269-278, Jul. 1986.*

CLAIMS:

17. An isolated nucleic acid molecule comprising a human tau gene sequence, said tau gene sequence having a mutation linked to a Tau pathology, wherein said mutation is in a region 13-16 nucleotides 3' of the exon 10 splicer donor site region of said tau gene sequence.

19. A method for determining a diagnosis, prognosis or risk of a Tau pathology in a patient, said method comprising detecting a tau

gene mutation in genomic DNA of said patient, wherein said mutation is in an exon of said tau gene or in a region 13-16 nucleotides 3' of the exon 10 splice donor site of said tau gene, wherein said mutation in said exon results in a deletion of amino acid 280, a threonine residue at amino acid 257, a valine residue at amino acid 272, a leucine residue or a serine residue at amino acid 301, an arginine residue at amino acid 389, or a tryptophan residue at amino acid 406 of a human Tau polypeptide, and wherein said mutation is associated with said Tau pathology.